Troubleshooting Guide for Transfection

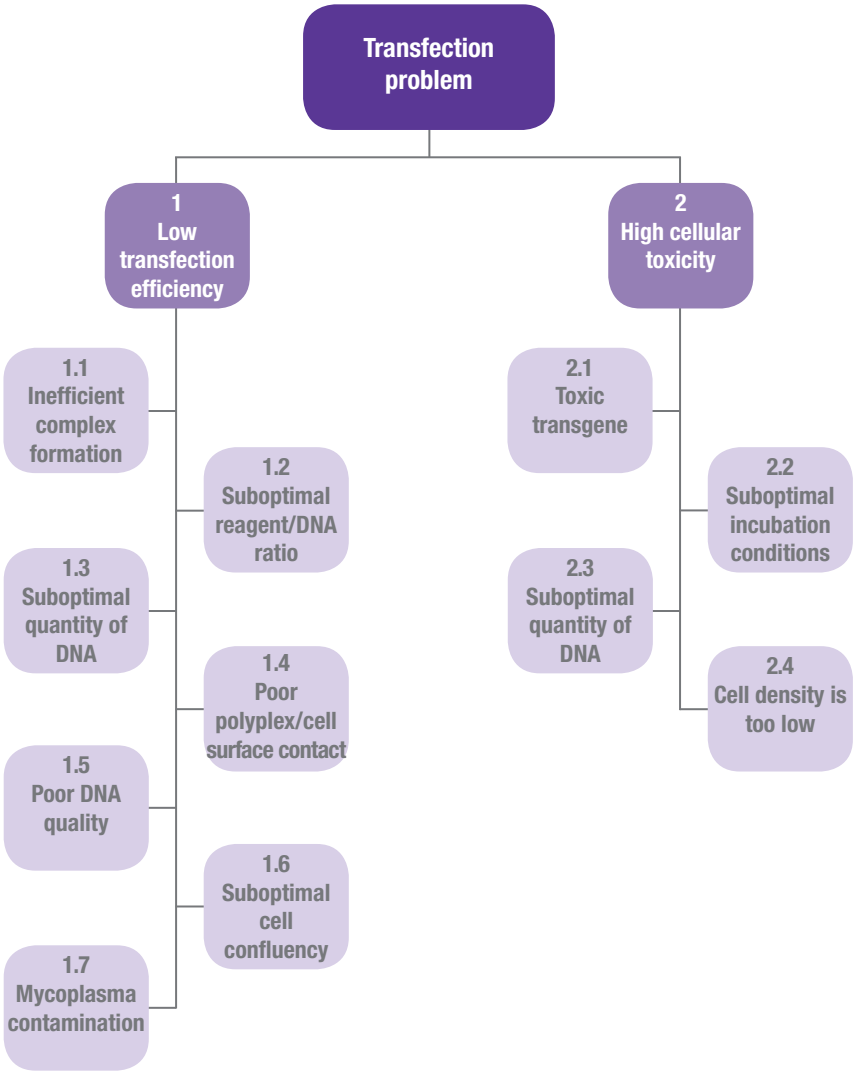

Table 11.6. Troubleshooting Guide for Transfection.

Problem	Possible cause and recommended solution
1. Low transfection efficiency	**1.1. Inefficient complex formation.** Always vortex the mixture immediately after the addition of the reagent to DNA. **1.2. Suboptimal reagent/DNA ratio.** Optimize the quantity of transfection reagent added to the fixed amount of DNA. **1.3. Suboptimal quantity of DNA.** Optimize the amount of DNA used for transfection. Keep the transfection reagent/DNA ratio constant **1.4. Poor polyplex/cell surface contact.** Centrifuge the culture plates, if it will not harm the cells. Caution – centrifugation may harm some primary cells. **1.5. Poor DNA quality.** Use high quality DNA with an A_{260}/A_{280} ratio greater than 1.8. **1.6. Suboptimal cell confluency.** Optimize cell plating conditions. Ensure that adhered cells are 50-70% confluent at the time of transfection. Ensure that suspension cells are in logarithmic growth phase at the time of transfection. **1.7. Mycoplasma contamination.** Mycoplasma infection in cell culture often results in poor and/or non-reproducible transfection. Regularly check your cells for mycoplasma infection.
2. High cellular toxicity	**2.1. Toxic transgene.** Verify if the expressed transgene is toxic **2.2. Suboptimal incubation conditions.** Reduce incubation time of the polyplexes with the cells. Replace the transfection mixture 3-6 hours later with fresh growth medium. **2.3. Suboptimal quantity of DNA.** Reduce the quantity of DNA used for transfection **2.4. Cell density is too low.** Increase the plating density of cells used for transfection.